

Influence of Preparation Conditions on the Physical Properties of Zein Films

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ABSTRACT: Zein is a hydrophobic protein produced from maize. Biodegradable zein films without additional reagents were prepared using various controlled drying conditions. The zein films were transparent. Mechanical properties (tensile strength and puncture strength), gas permeability, and water vapor permeability (WVP) of the zein films were measured. The tensile strengths of the zein films were between 7 and 30 MPa and the puncture strengths between 37 and 191 MPa. The zein films had higher oxygen permeability than carbon dioxide permeability. The lowest WVP of the zein film was 0.012×10^{-9} g·m/m²·s·Pa. We found differences in the WVP between the sides of the zein films; i.e., the air side of the zein film had a higher WVP than the basal side of the zein film when the films were exposed to high humidity during testing. This indicates a relationship between the WVP of the zein film and the contact angle of the zein film. The mechanical properties of the zein film depended on the drying conditions during preparation. Zein films with various useful physical mechanical properties were produced.

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In recent years, there has been considerable interest in biodegradable materials as part of a solution to environmental problems. Biodegradable materials made from natural materials, especially those based on starch, have attracted worldwide interest and a desire to expand their nonfood use, in part owing to their low cost. Despite receiving much interest, starch-based materials have failed to gain widespread use because they deteriorate very easily when subjected to a high-moisture environment. Therefore, many researchers have tried to produce starch-based biodegradable materials subjected to chemical modifications such as acetylation and/or the addition of natural or chemical plasticizers in starch (1,2).

Proteins from soybeans, milk, wheat, and corn have also been used to make biodegradable materials. Zein, the water-insoluble corn prolamin, provides an alternative to starch as a base for the production of biodegradable materials (3–5). This natural protein is found in gluten meal, a by-product of corn wet-milling. Zein can be transformed into a transparent, flexible and water-resistant film by dissolving the zein into a hydratable organic solvent such as ethyl alcohol (ethanol) or

acetone, and then drying. However, films prepared by using aqueous ethanol disintegrate when they come into contact with water, whereas films produced by using aqueous acetone have good water resistance (6). Additionally, zein molecules in aqueous acetone form the basic small aggregates that then grow into large aggregates relatively quickly, leading to the formation of the zein film structure more quickly than in aqueous ethanol (7). Swallen (8), one of the first proponents for the use of zein as an industrial protein, suggested that it had a variety of applications in food packaging materials and could form the basis of films for wrapping food, and that it had a number of nonfood uses as well. Biodegradable materials produced from zein have been studied by some researchers. Yamada *et al.* (6,7) reported on the manufacture of zein film using an aqueous acetone solution and on the physical properties of the resultant film. They found that zein film produced by using an aqueous acetone solution was water-resistant. Lai *et al.* (9–11) measured the tensile properties, water absorption, and surface structure of the zein film and the effects of adding plasticizers to the zein film. They reported that zein resin films showed good tensile and water barrier properties that were maintained through environmental humidity levels from 50 to 98% RH. Santosa and Padua (12) investigated the effect of oleic acid plasticization on the thermal behavior of rolled zein sheets with DSC. DSC endotherms were attributed to melting of free oleic acid or the dissociation of zein–oleic acid bonds. Plasticized sheets contained bound and free oleic acid. However, the drying conditions of the zein film were not accurately controlled.

Previously, we reported (13) on the surface morphology of the zein film, in which we found a relationship between the surface structure and the contact angle. Zein films with projections smaller than 200 nm in base diameter on the surface had a high contact angle ($>70^\circ$). In this study we produced films without additional reagents, and used a variety of controlled drying conditions and production methods. Our objective was to measure tensile strength and puncture strength, gas permeability, and water vapor permeability of the zein films produced under the various conditions.

EXPERIMENTAL PROCEDURES

Reagents. Purified α -zein (Showa ZeinTM; 96.3% protein, 3.4% water) was supplied by Showa Sangyo Co. Ltd., Tokyo, Japan. The molecular weight of α -zein (Z19) is 19,000 Da.

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Ethyl alcohol (ethanol), acetone, and calcium chloride (experimental grades) were obtained from Wako Pure Chemical Industries Ltd. (Osaka, Japan).

Preparation of the zein film. Zein powder (1.0 g) was dissolved in either 10 mL of 80% (vol/vol) aqueous ethanol or 70% (vol/vol) aqueous acetone and heated at 50°C for 10 min. The zein film was cast by pouring all of the zein solution onto a level, 200 cm², smooth polyethylene sheet using an auto-casting machine (Automatic Applicator type A; Toyoseki Co. Ltd., Tokyo, Japan). The solution was spread evenly and allowed to dry for 4 to 5 h at temperatures ranging from 30 to 45°C and between 5 and 90% RH in a controlled environmental chamber. The resultant film was then peeled intact from the casting surface. After drying, the zein films were kept at room temperature and at the same RH used during the zein film preparation. Specimens of the zein films were stored in a desiccator with silica gel for 24 h at room temperature before use. Sample codes, for example A-10-35-05, denoted the preparation conditions of the zein film type. The first character indicated whether an acetone (A) or an ethanol (E) solution was used. The first number was the initial concentration of the zein (%), the second number was the drying temperature (°C), and the last number was the drying RH (%).

Tensile strength and elongation. Tensile strength of the zein films was measured by using a rheometer (NRM-3002D; Fudoh Kogyo Co., Ltd, Tokyo, Japan). Samples were cut from the zein films by using a standard sample cutter (JIS Z1702; Rheotech, Tokyo, Japan). The tensile strength of the zein film was measured by pulling at a constant rate of 2 cm/min at room temperature. The elongation of the zein film was calculated by using the following expression:

$$E = 100 \frac{L_1 - L_0}{L_0} \quad [1]$$

where E = elongation at break (%), L_0 = initial length of film (mm), and L_1 = length at break (mm).

Puncture strength. The zein film was cut into 3 × 3 cm squares. The specimen was pasted onto a sample stage by using double-sided tape. The puncture strength of the zein film was measured by using a rheometer (NRM-3002D; Fudoh Kogyo Co., Ltd.) and a puncture steel bar (2 mm diameter) at a load constant rate of 2 cm/min at room temperature.

Gas permeability. A 19.625 cm² diffusion cell was used to measure the permeability of both the zein film and the polyethylene film (thickness, 20 μm; model DP-1; Sekisui Chemical Co., Ltd., Tokyo, Japan), as a control, to pure oxygen (O₂) and pure carbon dioxide (CO₂) by using the Gas Permeability Tester (Gasperm-100; JASCO Corporation, Tokyo, Japan). The sample (5 cm diameter) was placed in the diffusion cell at 24°C. The zein film was used to divide the diffusion cell into two sections. One side of the zein film was subjected to 0.05 MPa of pressure. The exception was sample E-35-35-05, which was subjected to 0.1 MPa of pressure while measuring CO₂ permeability because it could withstand a higher pressure. The pressure on the other side of the zein film was 0 MPa. Both a silicon gun and a Teflon filter were placed on the zein film to prevent gas leakage. The measuring area of the zein film was 0.785 cm².

Water vapor permeability (WVP). The WVP of the zein films was measured by using the Japanese Industrial Standard method, JIS (K7129: 1992). In this method the zein film was cut to 7 cm in diameter, and the film was used to seal a testing cup containing calcium chloride. This cup was placed in a controlled chamber at 313 K (40°C) and 90% RH. The weight of the cup was measured intermittently at intervals of 24 h, up to 96 h. WVP of the zein films was calculated as follows:

$$\text{WVP} = \frac{WL}{tAP} \quad [2]$$

where WVP = water vapor permeability (×10⁻⁹ g·m/m²·s·Pa), W = increase in cup weight (g), L = thickness of film (m), t = measuring time (s), A = measuring area (m²), and P = difference in pressure between outside of the cup and inside of the cup (Pa).

Data analysis. Data for properties of zein film were obtained from eight replicates and analyzed using Duncan's multiple range tests.

RESULTS AND DISCUSSION

Tensile strength and elongation. The zein films made from acetone solution (zein acetone films) had tensile strengths of about 21 MPa. The different preparation conditions used in making zein acetone films had no significant effect on the tensile strength (Fig. 1). On the other hand, the tensile strength of zein ethanol films ranged from a minimum of 7.1 MPa (E-10-35-90) to a maximum of 30.3 MPa (E-10-45-05). The tensile strength of the zein ethanol films depended on the drying conditions. The tensile strength of the zein ethanol films increased with increasing drying rate. E-10-45-05, with the fastest drying rate, had the highest tensile strength, and E-10-35-90, with the slowest drying rate, had the lowest tensile strength. Although zein ethanol films had various tensile strengths (Fig. 1), there was no variation in elongation (almost 4%), in comparing the tensile strengths of the zein films.

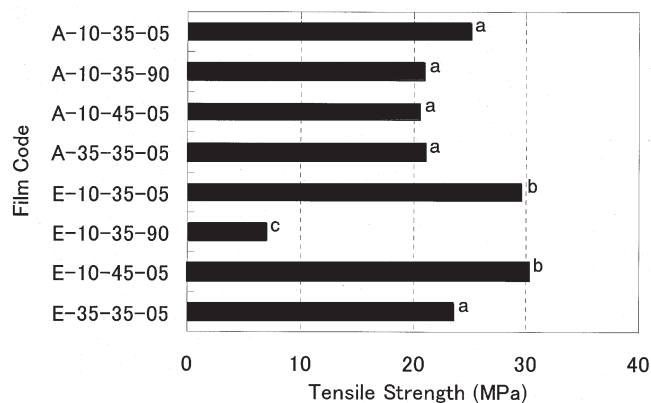


FIG. 1. Tensile strength of the zein films. Values followed by different letters are significantly different at $P < 0.05$ using Duncan's multiple range tests. Sample codes denote preparation conditions. A indicates acetone, and E indicates ethanol. The first number is the initial concentration of zein (%), the second is the drying temperature (°C), and the last number is the drying RH (%).

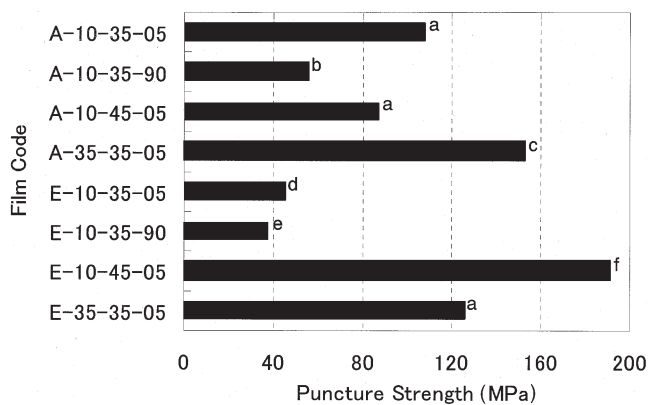


FIG. 2. Puncture strength of the zein films. Values followed by different letters are significantly different at $P < 0.05$ using Duncan's multiple range tests. For codes see Figure 1.

Puncture strength. Among zein acetone films, A-35-35-05 had the greatest puncture strength at 152.8 MPa (Fig. 2). The zein acetone film cast in 5% RH had a greater puncture strength than the zein acetone film cast in 90% RH. This result may be attributable to the absorption of water during drying by the zein acetone film cast in 90% RH. Puncture strength of the zein ethanol films increased with lower RH during drying and/or higher zein concentration (Fig. 2). The greatest puncture strengths were obtained at 125.7 MPa for E-35-35-00 and 191.3 MPa for E-10-45-05.

Gas permeability. The lowest and highest oxygen (O_2) permeabilities of the zein film were 1.93×10^{-9} (E-10-45-05) and $90.7 \times 10^{-9} \text{ cm}^3 \cdot \text{cm} / \text{cm}^2 \cdot \text{s} \cdot \text{cm Hg}$ (A-10-35-90), respectively (Fig. 3). On the other hand, the lowest and highest carbon dioxide (CO_2) permeabilities of the zein film were $0.30 \times 10^{-9} \text{ cm}^3 \cdot \text{cm} / \text{cm}^2 \cdot \text{s} \cdot \text{cm Hg}$ (E-10-35-05) and $452.9 \times 10^{-9} \text{ cm}^3 \cdot \text{cm} / \text{cm}^2 \cdot \text{s} \cdot \text{cm Hg}$ (A-35-35-05), respectively. In synthetic polymers such as a polyethylene film, CO_2 permeability is higher than O_2 permeability (14). However, the zein films, i.e., A-10-35-90, have a higher O_2 permeability than CO_2 permeability. O_2 and CO_2 permeabilities of A-10-35-90 were 90.7 and 9.15 ($\times 10^{-9} \text{ cm}^3 \cdot \text{cm} / \text{cm}^2 \cdot \text{s} \cdot \text{cm Hg}$), respec-

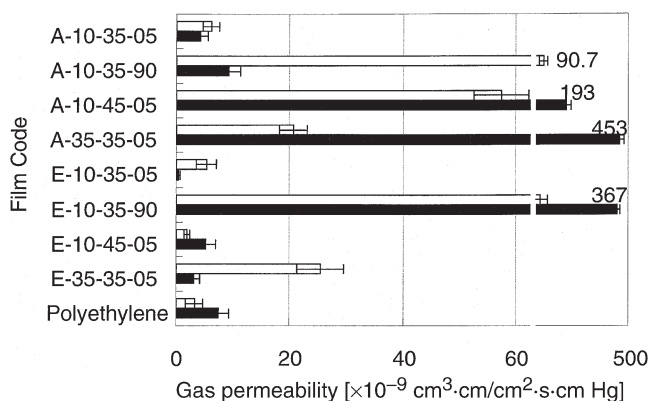


FIG. 3. Gas permeability of the zein films. □: O_2 permeability. ■: CO_2 permeability. Values of O_2 and CO_2 permeabilities for each of the zein films and for the polyethylene film are significantly different at $P < 0.05$ using Duncan's multiple range tests. Bars represent SE. For codes see Figure 1.

tively. The ratio of O_2 permeability to CO_2 permeability of the 10% zein acetone films increased with increasing RH during drying (Fig. 4). Hydrophobic interactions and/or hydrogen bonding has an important role in protein folding. The drying rate of zein solutions depends on environmental conditions during the preparation process of the zein film. The drying rate of zein acetone solutions in low humidity is faster than the drying rate of zein acetone solutions in high humidity. Thus, because zein molecules are insoluble in water, as the movement of the zein molecules decreases, zein molecules are apt to become the associated zein body. Conversely, the drying rate of zein acetone solutions in high humidity is low, making it easier to keep the zein molecules moving during drying. Therefore, the volume of the associated zein body is different under different RH. Gaps between associated zein bodies when high-humidity conditions are used in the preparation process are larger than gaps between associated zein bodies using low-humidity conditions in the preparation process, resulting in the gas permeability of high-RH zein films being highest. Yamada *et al.* (6) reported that associated zein bodies have polarity. As carbon dioxide has polarity, its permeability does not change, but the permeability of oxygen increases from low humidity to high humidity. Mujica-Paz and Gontard (15) reported that the permeability of CO_2 and O_2 in wheat gluten protein films depends on environmental humidity conditions. They suggested that the protein network is transformed because the water molecules in high humidity conditions affect the amide bonding of proteins. Thus, the gas permeability of soy protein films, including the dissolution coefficient and the diffusion coefficient, is changed by the altered protein network. In zein films, the protein network is transformed in the high-humidity preparation process, decreasing both the dissolution coefficient and the diffusion coefficient of CO_2 ; thus, the dissolution coefficient and the diffusion coefficient of carbon dioxide are lower than those for oxygen. We found that zein films have selective gas permeability depending on the drying conditions, especially the RH.

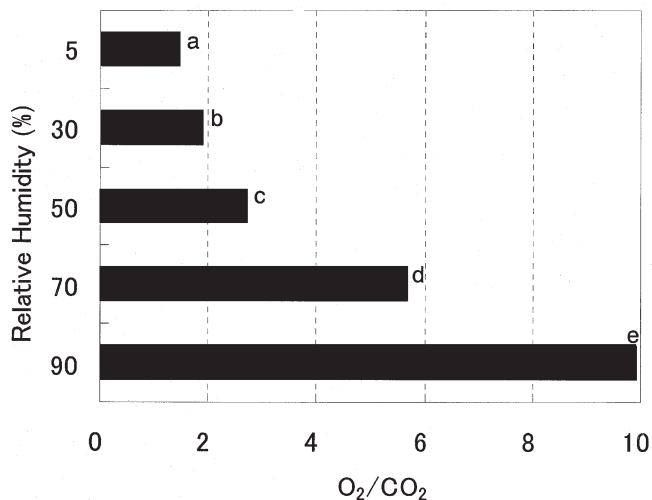


FIG. 4. The ratio of O_2 permeability to CO_2 permeability of the 10% zein acetone films. Values followed by different letters are significantly different at $P < 0.05$ using Duncan's multiple range tests.

The ratio of O₂ and CO₂ permeability of the zein film can therefore be controlled by means of the drying conditions during film formation; thus, the atmospheric conditions inside packages of fruits and vegetables can be regulated.

WVP. There was no large difference in WVP between the zein acetone films and the zein ethanol films (Fig. 5). The WVP of both films had between 0.01×10^{-9} and 0.09×10^{-9} g·m/m²·s·Pa. But there was a marked difference in the WVP between the two surfaces of both these films. When the side of the zein film exposed to the air during the casting process (air side) was set on the outside of the cup, the zein film had a higher WVP than when the side of the zein film in contact with the polyethylene sheet during the casting process (basal side) was set on the outside. The results of these experiments suggest a correlation between the WVP of the zein film and the contact angle of the zein film. Therefore, we can deduce that the zein film with a high contact angle has a low WVP. WVP depends on the surface properties and internal structure of the zein film. In zein films water has a greater tendency to adhere to films with a low contact angle than to films with a high contact angle. We assume that zein molecules have both a hydrophilic surface and a hydrophobic surface (Fig. 6). A hydrophobic polyethylene sheet was used for zein film preparation. Initially, the hydrophobic part of the zein molecule was in contact with the sheet and there were interactions between the solution and the hydrophobic or the hydrophilic parts of each molecule. The zein molecules have a tendency to keep a hydrophobic bonding between the acetone molecule and hydrophobic part of zein molecules in the solution. However, because the evaporation rate of acetone is higher, the zein molecules were attracted by acetone during drying, making the air side of the zein acetone film comparatively hydrophobic. On the other hand, ethanol molecules have both a hydrophobic and a hydrophilic functional group. In solution, the binding ability between ethanol molecules and the hydrophobic part of the zein molecules decreases. After drying, the surface hydropho-

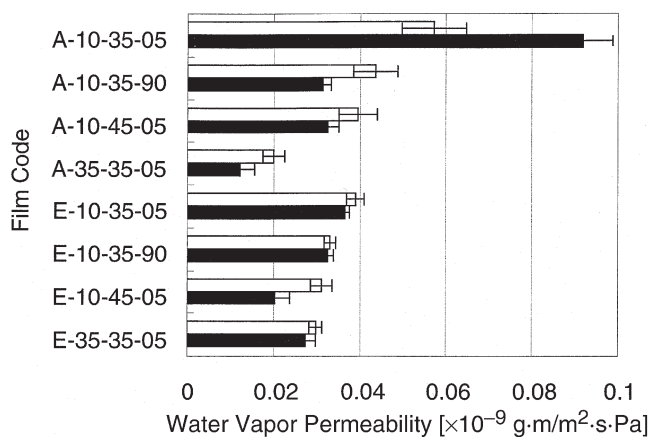


FIG. 5. Water vapor permeability (WVP) of the zein films. □: WVP of the air side of the zein film was turned upward on the testing cup. ■: WVP of the basal side of the zein film was turned upward on the testing cup. Values between the air side and basal side of zein acetone films are significantly different at $P < 0.05$ using Duncan's multiple range tests. Bars represent SE. For codes see Figure 1.

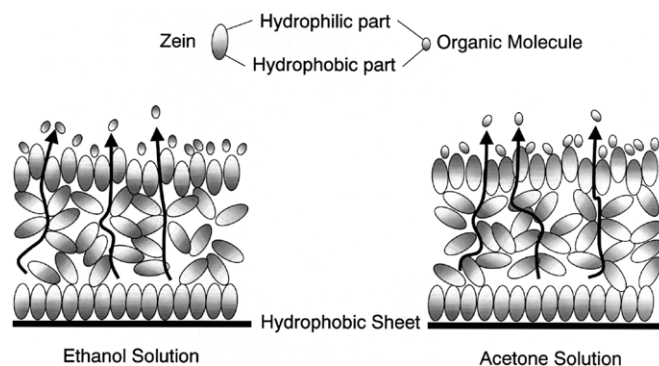


FIG. 6. Schematic representation of the structure of the aggregated zein molecules in the organic solution. The hydrophobic part of the zein molecules touched the hydrophobic sheet. The surface property of the side of the zein film exposed to the air during the casting process depends on the organic solvent.

bicity of the zein ethanol films decreased. In the initial adhesion of water to the zein film surface, the air side of the zein film has higher adhesion properties than the basal side of the zein film. Therefore, the basal side of the zein films had a lower WVP than the air side of the zein film. When the zein film is used for food packaging, choosing the side with a high WVP or low WVP must be considered.

Zein films that were homogeneous and transparent were made from corn hydrophobic protein (α -zein) in either acetone or ethanol solutions. The physical properties of the zein film depended on the drying rate during preparation. We elaborated on the effects of drying temperature and/or RH on tensile strength, puncture strength, gas permeability, and water vapor permeability. The differences in physical properties of the different zein films were most likely caused by variation in the internal microstructure of the zein films. We expect that it will be possible to make zein films with various useful physical properties by controlling the drying conditions.

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